Ind. J. Pharm. Edu. Res., 2026; 60(3s):s1203-s1214.
https://www.ijper.org

Original Article

Extraction, Phytochemical Analysis, and Antioxidant Activity of *Adhatoda vasica* Leaves

Hameed Fathima Kamal[1], Jaideep Mahendra[1,*], Ashok Kumar Pandurangan[2,*]

[1]Department of Periodontics, Meenakshi Ammal Dental College and Hospital, Maduravoyal, Chennai, Tamil Nadu, INDIA.
[2]School of Life Sciences, B.S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Background: *Adhatoda vasica* is a well-known medicinal plant with diverse therapeutic properties, widely used in traditional medicine. Its phytochemical composition and antioxidant potential warrant detailed investigation. **Objectives:** This study aimed to evaluate the extraction efficiency, phytochemical profile, and antioxidant activity of aqueous and ethanolic leaf extracts of *Adhatoda vasica*. **Materials and Methods:** Aqueous and ethanolic extracts of *Adhatoda vasica* leaves were prepared using standard extraction procedures. Phytochemical screening was conducted to detect the presence of bioactive compounds such as saponins, flavonoids, tannins, and alkaloids. Liquid Chromatography-Mass Spectrometry (LCMS-MS) analysis was performed to identify bioactive constituents. Antioxidant activity was assessed using DPPH and ABTS radical scavenging assays, with naringenin used as a positive control. **Results:** Phytochemical analysis confirmed the presence of key secondary metabolites in both extracts. LCMS-MS analysis identified a total of 153 bioactive compounds, with a greater number detected in the ethanolic extract compared to the aqueous extract. Antioxidant assays demonstrated that the ethanolic extract exhibited significant free radical scavenging activity in a concentration-dependent manner, comparable to the standard naringenin. **Conclusion:** The findings indicate that *Adhatoda vasica* leaves are a rich source of bioactive compounds with potent antioxidant activity. The ethanolic extract, in particular, shows promising potential for the development of natural therapeutic agents targeting oxidative stress-related disorders. Further studies are needed to explore the pharmacological applications of these compounds.

Keywords: ABTS, *Adhatoda vasica*, Antioxidant, DPPH, LCMS-MS.

Correspondence:
Dr. Jaideep Mahendra
Professor and Head, Department of Periodontics, Meenakshi Ammal Dental College and Hospital, Maduravoyal, Chennai-600095, Tamil Nadu, INDIA.
Email: drjaideep.perio@madch.edu.in

Dr. Ashok Kumar Pandurangan
Associate Professor, School of Life Sciences, B. S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai-600048, Tamil Nadu, INDIA.
Email: panduashokkumar@gmail.com

Received: 12-02-2026;
Revised: 06-04-2026;
Accepted: 22-05-2026.

INTRODUCTION

Medicinal plants contain numerous biologically active compounds that are helpful in the treatment of various diseases and improving life.[1] They are possessed to have various properties, such as antioxidant, anti - inflammatory, anti-cancer, anti-diabetic, anti-helminthic properties. *Adhatoda vasica*, commonly known as the Malabar nut tree, is a part of the Acanthaceae plant family[2] is a notable plant in traditional medicine because of its wide range of pharmacological properties. The roots, leaves, and flowers are the active parts of plants that are used to treat various diseases. The leaves and roots contain several key alkaloids, such as quinazoline alkaloids, vasicine, vasicinone, vasicinolone, and vasicol, in combination with a compound called adhatoda acid. *Adhatoda vasica* extract is used for a variety of health conditions due to its broad spectrum of effects as a sedative, expectorant, antispasmodic, anthelmintic, bronchial antiseptic, bronchodilator and anti - cancer drug. It is also prescribed for bleeding due to idiopathic thrombocytopenic purpura, local bleeding due to peptic ulcer, piles, menorrhagia and also used in treatment of pyorrhea and in bleeding gums.[3] Its wide-ranging effects make it a valuable herb in traditional medicine.

Phytochemicals of plant extracts exhibit various bioactivities, such as antimutagenic, anticarcinogenic, antioxidant, antibacterial, and anti-inflammatory properties.[4] The main bioactive components in plants are alkaloids, tannins, flavonoids, phenolic compounds, terpenoids, and Antioxidants; in particular, they play a crucial role in protecting the body from oxidative damage caused by free radicals and Reactive Oxygen Species (ROS). By neutralizing these harmful molecules, antioxidants can prevent cellular damage and reduce the risk of various chronic diseases.[5-10] In the present study, we aimed to extract and characterize the phytoconstituents of *Adhatoda vasica* using LC-MS and further study its antioxidant properties.

DOI: 10.5530/ijper.20264592

MATERIALS AND METHODS

Collection of *Adhatoda vasica*

Adhatoda vasica leaves were collected from a local market in Chennai (India) in November 2020. It was authenticated by The Siddha Central Research Institute (Centre Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India, Anna Govt. H Hospital Campus, Arumbakkam, Chennai 600106), with authentication certificate number 318.25052201.

Preparation of crude extracts

Fresh and healthy leaves of *Adhatoda vasica* were sourced and shade-dried for a week. Once the leaves were completely dried, they were cleaned and ground to obtain fine powder. Both aqueous and ethanolic extracts were prepared by dissolving the leaf powder in water and ethanol at a 1:2 ratio and left overnight. The following day, the mixtures were filtered using Whatman filter paper and the extracts were stored at 4° C until further analysis.

Phytochemical analysis

The prepared *Adhatoda vasica* extracts were investigated to determine the presence of saponins, steroids, terpenoids, phytosterols, flavonoids, tannins, phenols, phenolic flavonoids, and alkaloids, according to the methods of Sadasivam.[11] The positive results of these tests were determined by observing precipitate formation or any color change.

Saponins

Approximately 2 mL of distilled water was mixed with 1 mL of *Adhatoda vasica* extract. The mixture was mixed well for a few seconds and allowed to stand for 5-10 min. The presence of saponins was determined by the formation.

Terpenoids

Adhatoda vasica extract (1 mL) was added to an equal volume of concentrated sulfuric acid (H_2SO_4). Terpenoids were detected by the appearance of a reddish-brown color.

Steroids

Concentrated sulfuric acid (H_2SO_4) was added to *Adhatoda vasica* extract (0.5 mL) along with 1 mL of chloroform. The upper layer turned yellow, and the lower layer turned green and fluorescent. These color changes confirm the presence of steroids.

Phytosterols

Chloroform (1 mL) was added to an equal volume of *Adhatoda vasica* extract, followed by a few drops of H_2SO_4. The mixture was allowed to stand for a few minutes. The presence of a golden yellow tint indicates the presence of phytosterol.

Tannins

Freshly prepared 5% Ferric Chloride ($FeCl_3$) was added to 1 mL of *the Adhatoda vasica* extract. The dark green or greenish-black color indicates the presence of tannin.

Flavonoids

A few drops of 10% Sodium Hydroxide (NaOH) were added to 1 mL of *Adhatoda vasica* extract. The presence of flavonoids was indicated by a brown precipitate.

Phenol

Phenol was detected by adding a few drops of alcoholic $FeCl_3$ solution to 2 mL of *the Adhatoda vasica* extract. The formation of a bluish color suggests the presence of phenols.

Phenolic Flavonoids

A few drops of freshly prepared 10% lead acetate were added to 1 mL of *Adhatoda vasica* extract. The brown precipitation indicated the presence of phenolic flavonoids.

Alkaloids

Mayer's reagent (1 mL) was added to 1 mL of *the Adhatoda vasica* extract. The presence of alkaloids was confirmed by formation of a white precipitate.

Phenolic Flavonoids

A few drops of freshly prepared 10% lead acetate were added to 1 mL of *Adhatoda vasica* extract. The brown precipitation indicated the presence of phenolic flavonoids.

LC-MS-MS Analysis

An Acquity H-class UPLC (Waters Corporation, Milford, MA, USA) was employed, which had an integrated vacuum degasser, automatic sample manager (Serial # C10UPA554M, Waters Corporation, Singapore), ultra-performance binary solvent manager (Serial # C10UPB081A, Waters Corporation, Singapore), and injection volume range of up to 100 µL with an optional extension loop. A C18 stationary phase (Accucore C18, 50 x 4.6 mm, 2.6µ) was used for chromatographic separation. Xevo G2-XS QToF (Serial # YFA1548, Waters Corporation, Wilmslow, UK) was employed for Mass Spectrometric (MS) detection.

The sample was injected in a volume of 5 µL. The column oven temperature was maintained at the optimal level throughout the chromatographic run (22°C). For MS detection, a positive-polarity electrospray ionization (ESI) source was used. The optimal instrument and acquisition parameters were 50 L/h. cone gas (nitrogen) flow, 750 L/h; desolvation gas (nitrogen) flow; 450°C probe temperature; sampling cone voltage; 150°C source temperature; source offset voltage, 80 V; collision energy ramp, 6-50 eV (argon, collision gas); and mass range, 50-2000 m/z. To

acquire and process the data, Waters Corporation's Mass Lynx software (V4.1, Milford, MA, USA) was used.

Total Phenols, and Tannins Content

Total phenols were determined using the colorimetric method of Folin-Ciocalteu,[12] An aliquot (0.5 mL) of diluted ethanol extract was mixed with deionized water (35 mL) and 2.5 mL of Folin-Ciocalteu reagent; 3 min of incubation, and sodium carbonate solution (20% in water) (5 mL). The solution was incubated at 70°C for 20 min and brought to a volume (50 mL) with deionized water. Absorbance was read at 750 nm with a UV-vis spectrophotometer (CARY 50 VARIAN, Amsterdam, The Netherlands). The results are expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW). For calibration curve, gallic acid concentrations were used between 0.05-0.5 mg/mL (R^2=0.99).

Tannins were determined according to Fadda and Mulas,[13] 4 mL of the diluted extract was mixed with ethanol (2 mL) and a vanillin solution (1% vanillin in 70% H_2SO_4). After 30 min of incubation at room temperature, absorbance was measured at 500 nm. The results are expressed as mg of Catechin Equivalent (CE)/g DW based on the calibration curve (0.05-0.5 mg/mL catechin, R^2=0.99).

Antioxidant Activity

DPPH Radical-Scavenging Activity

The antioxidant activity of *Adhatoda vasica* extract was analyzed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay method proposed by Katalinic *et al.*,[14] Methanolic DPPH was diluted to a concentration of 1×10^{-4} M. Dilutions of the samples were prepared in methanol ranging from 100 to 500 µg/mL. A blank sample containing only methanolic DPPH dilution was prepared. A positive control was prepared using naringenin at concentration of 10µg/mL - 100µg/ml. One milliliter of each sample solution was added to be tested in a series of cuvettes. The prepared DPPH solution (2 mL) was added to the prepared DPPH solution. Control cuvettes were prepared containing only the DPPH reagent. All cuvettes were placed in the dark at room temperature for 30 min to allow the reaction to proceed. After incubation, the absorbance of each cuvette was measured at 517 nm using a UV-vis spectrophotometer. The absorbance values for each sample and control were recorded. Percentage inhibition was calculated using the following formula:

Percentage of inhibition=[(Abs_control - Abs_sample) / Abs_control] × 100

The obtained data were analyzed to determine the antioxidant activity of the samples relative to that of the controls. The percentage inhibition of the samples was compared with that of the positive controls to assess antioxidant potential.

ABTS Radical Scavenging Activity

ABTS [2,20 -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] radical scavenging activity was measured by dissolving ABTS powder in PBS to prepare a 7 mM ABTS stock solution. Potassium persulfate ($K_2S_2O_8$) was added to the ABTS solution at a final concentration of 2.45 mM. The mixture was incubated in the dark at room temperature for 12-16 hr to generate ABTS radical cation ($ABTS^{\bullet+}$). Before each assay, the $ABTS^{\bullet+}$ solution was diluted with methanol to an absorbance of approximately 0.70 at 734 nm using a UV-visible spectrophotometer. The solution was allowed to equilibrate at RT. This procedure was performed to standardize the ABTS solution. Samples were diluted in methanol at appropriate concentrations. A blank containing methanol was also prepared. Positive controls were prepared with naringenin at known concentrations. In a series of cuvettes, 1 mL of each sample solution and 1 mL of the diluted $ABTS^{\bullet+}$ solution were added to each cuvette. All the cuvettes were incubated in the dark at room temperature for 6 min. After incubation, the absorbance of each cuvette was measured at 734 nm using a UV-visible spectrophotometer. The absorbance values for each sample and the control were recorded. Percentage inhibition was calculated using the following formula:

Percentage of inhibition=[(Abs_control - Abs_sample) / Abs_control] × 100

Statistical analysis

Statistical analyses were performed using SPSS 16.0 version. Statistical significance was set at $p<0.05$.

RESULTS

Phytochemical analysis

The phytochemicals, including tannins, flavonoids, saponins, phytosterols, terpenoids, phenols, alkaloids, and steroids, of various extracts from the leaves of *Adhatoda vasica* were screened and are depicted in Table 1.

All the phytochemicals were present in the aqueous extract. Saponin was absent in the ethanol extract, and other phytochemicals such as tannins, flavonoids, phytosterols, terpenoids, phenols, steroids, and alkaloids were present in both ethanolic and water extracts. In concordance with previous reports, the phytochemicals were confirmed in our analysis.[15,16]

Estimation of total phenols and tannins

Figure 1 shows the total phenol content and tannins in the aqueous and ethanolic extracts of *Adhatoda vasica.* It was observed that the total phenols increased in a dose-dependent manner in both the aqueous and ethanolic extracts of *Adhatoda vasica*, with the aqueous extract showing a higher amount of total phenols than the ethanolic extract (Figures 1A, B). We also observed that the tannins were estimated in the aqueous and ethanolic

extracts of *Adhatoda vasica* (Figures 1C, D). The aqueous extract *Adhatoda vasica* showed a higher amount of total phenols than the ethanolic extract. We conclude that the aqueous extract of *Adhatoda vasica* rich in total phenols and tannins. Mohammed *et al.*,[17] and Nandhini and Ilango[18] reported that the amounts of total phenols and tannins were nearly at the microgram level. In our results, we found that nearly similar levels of total phenols and tannins contributed to the strong antioxidant property of *Adhatoda vasica*.

LC-MS-MS Analysis

Liquid Chromatography with tandem mass spectrometry detected compounds of *Adhatoda vasica* (Tables 2 and 3 and Figures 2 and 3). In total, 153 compounds were identified in the leaf extract of *Adhatoda vasica*. The highest number of compounds was identified in ethanol extracts. The peaks were predominantly observed, and prominent compounds were identified in the ethanolic extract when compared to the aqueous extract. The results of the present study showed that both extracts of *Adhatoda vasica* contained bioactive compounds with anti-microbial, antioxidant, and anti-cancer properties. The active constituents and their retention times (RT), compound names, molecular formulas, and molecular weights are given in Tables 2 and 3.

Antioxidant activity

Antioxidant activity is determined by the free radical scavenging capacity or inhibition of oxidation by different biological mechanisms.

The DPPH assay is a simple and prominent method for evaluating the free radical scavenging ability. The hydrogen-donating capacity of the extracts was thought to be responsible for the

Table 1: Phytochemical analysis of *Adhatoda vasica* leaves.

Sl. No.	Phytochemical test	Aqueous extract	Ethanolic extract
1	Saponins	+	-
2	Terpenoids	+	+
3	Steroids	+	+
4	Phytosterols	+	+
5	Tannins	+	+
6	Flavonoids	+	+
7	Phenol	+	+
8	Alkaloids	+	+

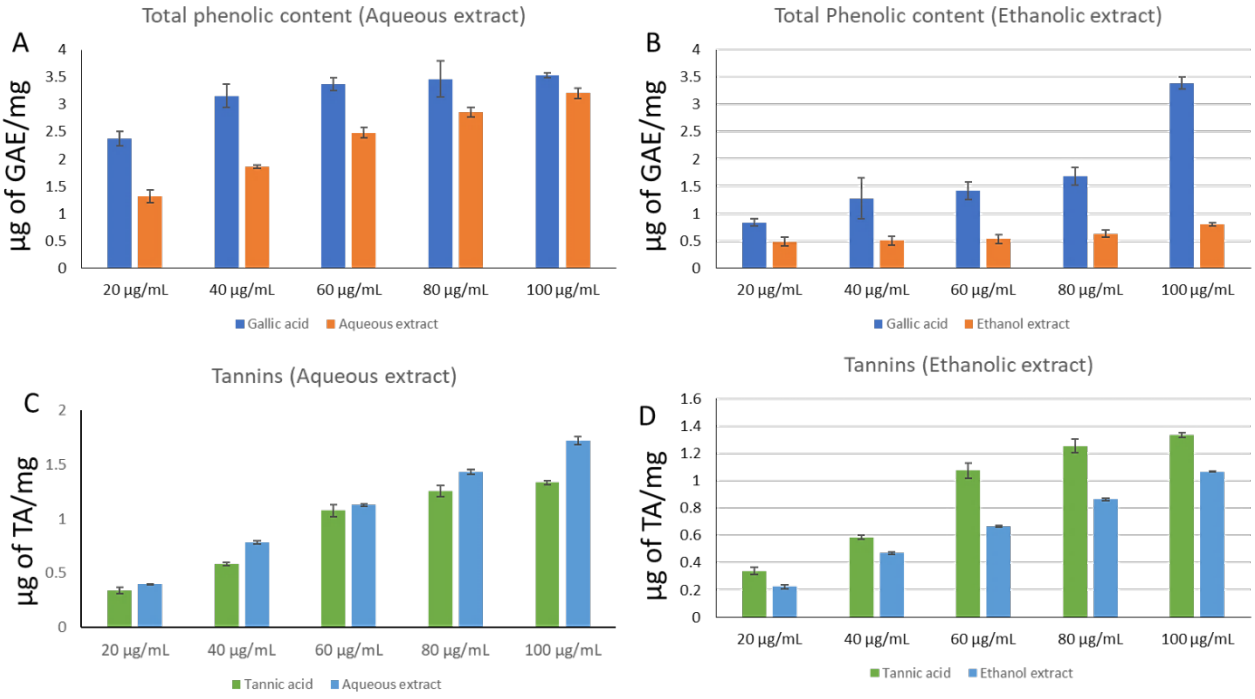

Figure 1: The estimation of total phenols and tannins in aqueous and ethanolic extract of *Adhatoda vasica*. The phenols and tannins were expressed µg/mL.

DPPH radical scavenging activity. The antioxidant compound reacts with the radical DPPH, which reduces to DPPH-H, which can be observed by the reduction in absorbance values. The *Adhathoda vasica* extracts exhibited DPPH scavenging effects in a concentration-dependent manner, as shown in Figure 4.

ABTS Assay

Similar to DPPH activity, ABTS was also performed in a dose-dependent manner, as shown in Figure 5. The positive control (naringenin) showed a dose-dependent increase in antioxidant activity, with higher concentrations resulting in a greater percentage inhibition of the ABTS radical. Similarly, the ethanol extract also exhibited antioxidant activity, with higher concentrations showing increased percentage inhibition compared to lower concentrations. On comparing the ethanol extract with the positive control, it was observed that at certain concentrations (25 µg and 75 µg), the antioxidant activity of the ethanolic extract was similar to or exceeded the antioxidant activity of Naringenin as shown in Figure 4.

DISCUSSION

The present study was conducted to identify the various bioactive compounds present in *Adhatoda vasica* ethanol and aqueous extracts using LC-MS-MS. Aqueous and ethanol extracts were taken for this study because they are considered prospective sources of antiviral, antitumor, and antimicrobial agents in allopathic medicine.[19] Phytochemical analysis also showed that both the aqueous and ethanol extracts of *Adhatoda vasica* had numerous types of major phytochemicals such as tannins, flavonoids, saponins, phytosterols, terpenoids, phenols, alkaloids, and steroids. These Phytochemicals can also exhibit various bioactivities, such as antimutagenic, anticarcinogenic, antioxidant, antibacterial, and anti-inflammatory properties.[20] These metabolites have numerous therapeutic benefits, and are widely used in the drug and pharmaceutical industries. Tannins and saponins are excellent anti-microbial agents, whereas flavonoids and polyphenols are antioxidant agents. Flavonoids are water-soluble antioxidants that scavenge free radicals.

Liquid Chromatography with tandem mass spectrometry was performed to detect the phytochemical constituents. LC-MS-MS analysis of aqueous and ethanol extracts revealed multiple peaks,

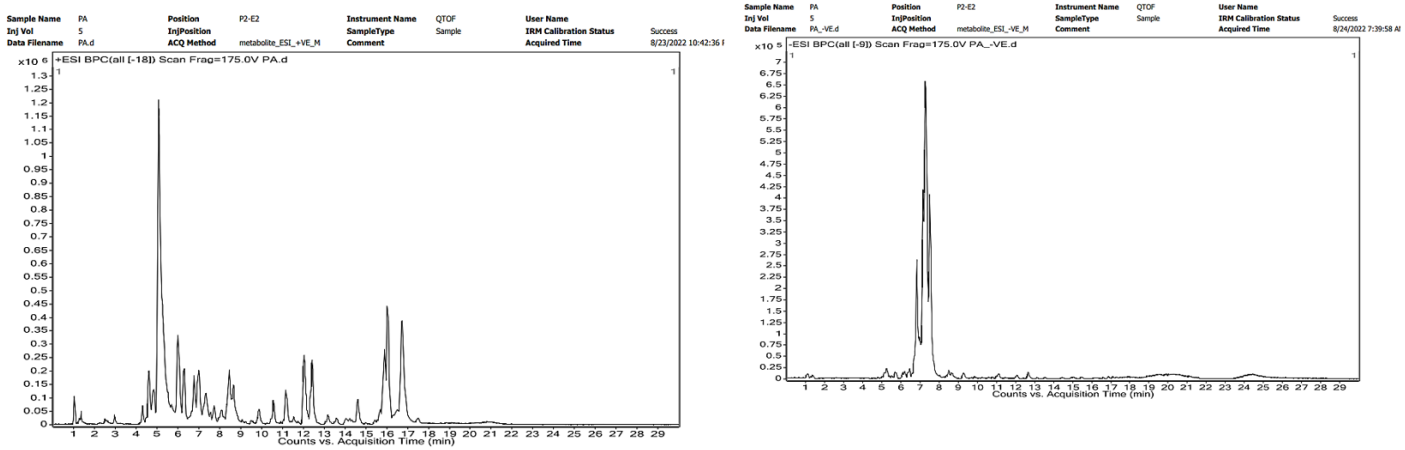

Figure 2: LCMSMS peaks of aqueous extract of *Adathoda vasica* leaves.

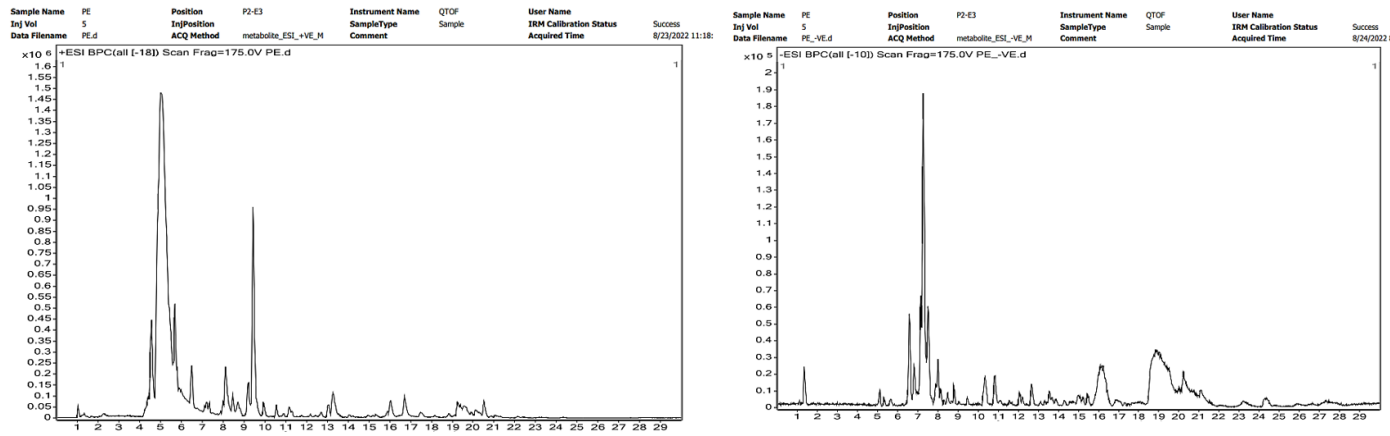

Figure 3: LC-MSMS peaks of Ethanol extract of *Adathoda vasica* leaves.

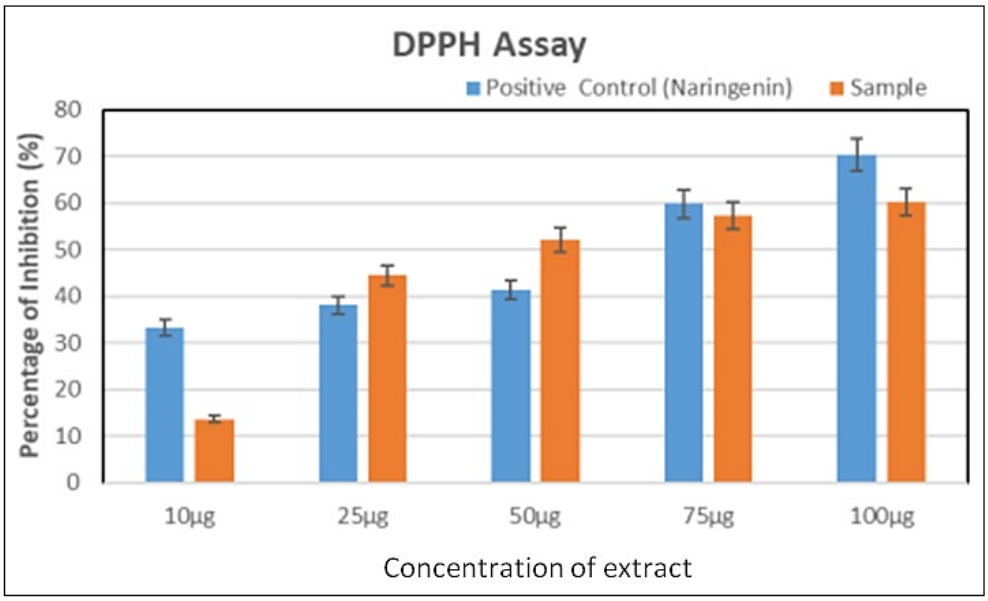

Figure 4: Shows the percentage of inhibition of ethanolic extract of *Adhatoda vasica* at different concentration using DPPH assay.

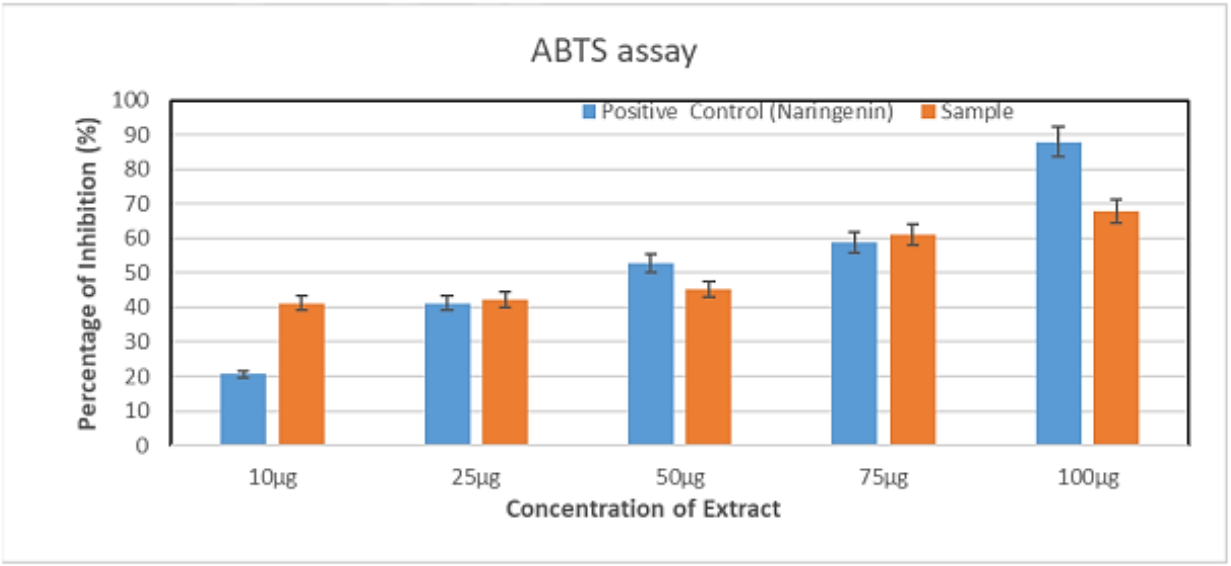

Figure 5: Shows the percentage of inhibition of ethanolic extract of *Adhatoda vasica* at different concentration using ABTS assay.

indicating the presence of numerous phytochemical compounds. A total of 153 compounds from aqueous and ethanol extracts were obtained from the leaves of *Adhatoda vasica*. 73 compounds were identified in the aqueous extract and 80 compounds were identified in the ethanol extract. The results clearly showed that more compounds were identified in the ethanol extract than in the aqueous extract. The outcome of the current study closely aligns with the research conducted by Kripasana and Xavier,[21] where the maximum number of chemical compounds was isolated from the polar solvent leaf extract of Adhatoda. Thus, screening for different phytochemicals of *Adhatoda vasica* can help in the detection of new active compounds.

Phenolic compounds and tannins are significant antioxidant constituents, primarily because of their capacity to neutralize free radicals through the donation of hydrogen atoms.[22,23] These molecules possess optimal structural features conducive to free radical scavenging. Various studies have demonstrated a linear correlation between the total phenolic and tannins and antioxidant capacity.[24,25]

Table 2: LC-MS-MS analysis of the Aqueous extract of *Adhathoda vasica*.

Sl. No.	RT	Compound	Molecular Weight	Molecular formula
1.	1.015	(+/-)-3-[(2-methyl-3- furyl)thio]-2-butanone	184.0571	$C_9 H_{12} O_2 S$
2.	4.282	(S)-3- [(Cyanophenylmethyl)amino]-3-oxopropanoic acid	218.0685	$C_{11} H_{10} N_2 O_3$
3.	4.549	L-N-(1H-Indol-3- ylacetyl)glutamic acid	304.1051	$C_{15} H_{16} N_2 O_5$
4.	4.683	L-Tryptophan	204.089	$C_{11} H_{12} N_2 O_2$
5.	4.85	(1xi,3S)-1,2,3,4-Tetrahydro-1-methyl-beta-carboline-1,3- dicarboxylic acid	274.0944	$C_{14} H_{14} N_2 O_4$
6.	5.064	L-1,2,3,4-Tetrahydro-betacarboline-3-carboxylic acid	216.0894	$C_{12} H_{12} N_2 O_2$
7.	5.118	Peganine	188.0946	$C_{11} H_{12} N_2 O$
8.	5.138	2-Amino-5-phenylpyridine	170.084	$C_{11} H_{10} N_2$
9.	5.191	2-Hydroxybiphenyl	170.0707	$C_{12} H_{10} O$
10.	5.269	N-Acetyl-D-tryptophan	246.1001	$C_{13} H_{14} N_2 O_3$
11.	5.5	4-Coumaroyl-2- hydroxyputrescine	250.1319	$C_{13} H_{18} N_2 O_3$
12.	5.657	7-(4-Hydroxyphenyl)-1-phenyl-4-hepten-3-one	280.1443	$C_{19} H_{20} O_2$
13.	5.727	Phenylalanyl-Alanine	236.118	$C_{12} H_{16} N_2 O_3$
14.	5.763	4-Hydroxyaminoquinoline N-oxide	176.0581	$C_9 H_8 N_2 O_2$
15.	5.782	N-Acetylserotonin	218.1049	$C_{12} H_{14} N_2 O_2$
16.	5.912	N-Feruloylglycyl-Lphenylalanine	398.1474	$C_{21} H_{22} N_2 O_6$
17.	6.038	Pheneturide	206.1053	$C_{11} H_{14} N_2 O_2$
18.	6.102	Morusin	420.156	$C_{25} H_{24} O_6$
19.	6.321	Myxochelin A	404.1603	$C_{20} H_{24} N_2 O_7$
20.	6.667	Kaempferol 3-rhamnoside 7- xyloside	564.1469	$C_{26} H_{28} O_{14}$
21.	6.671	epsilon-Viniferin	454.1368	$C_{28} H_{22} O_6$
22.	6.845	Nisoldipine	388.1652	$C_{20} H_{24} N_2 O_6$
23.	6.972	2-(4-Ethoxyphenyl)-5,6,7,8- tetramethoxy-4H-1-benzopyran-4-one	386.1372	$C_{21} H_{22} O_7$
24.	7.146	Gibberellin A105	330.1478	$C_{19} H_{22} O_5$
25.	7.153	4'-O-methyl-(-)-epicatechin-3'-O-beta-glucuronide	494.1438	$C_{23} H_{26} O_{12}$
26.	7.428	Albafuran C	580.171	$C_{34} H_{28} O_9$
27.	7.483	Flavonol 3-O-D-galactoside	400.1173	$C_{21} H_{20} O_8$
28.	7.568	Mycinamicin IV	695.4292	$C_{37} H_{61} N O_{11}$
29.	7.661	Tryptophyl-Tryptophan	390.1692	$C_{22} H_{22} N_4 O_3$
30.	7.78	Isolariciresinol 9'-O-alpha-Larabinofuranoside	492.202	$C_{25} H_{32} O_{10}$
31.	7.914	Cascaroside D	564.1802	$C_{27} H_{32} O_{13}$
32.	7.968	Famciclovir	321.1475	$C_{14} H_{19} N_5 O_4$
33.	8.003	Tryptophyl-Alanine	275.1292	$C_{14} H_{17} N_3 O_3$
34.	8.09	Physalin E	544.1951	$C_{28} H_{32} O_{11}$
35.	8.215	(6S)-dehydrovomifoliol	222.1243	$C_{13} H_{18} O_3$
36.	8.233	Dihydrocorynantheine	368.2124	$C_{22} H_{28} N_2 O_3$
37.	8.307	Aurasperone E	588.1739	$C_{32} H_{28} O_{11}$

Sl. No.	RT	Compound	Molecular Weight	Molecular formula
38.	8.444	(R)-Heraclenol 2'-(3- methylbutanoate)	388.1535	$C_{21}H_{24}O_7$
39.	8.582	Indacaterol	392.2095	$CH_{28}N_2O_3$
40.	8.775	N2'-Acetylgentamicin C1a	491.2969	$CH_{41}N_5O_8$
41.	8.845	Vomifoliol 9-[xylosyl-(1->6)- glucoside]	518.2341	$C_{24}H_{38}O_{12}$
42.	9.499	Icaceine	375.2411	$C_{22}H_{33}NO_4$
43.	9.57	2-Methoxyestrone 3-sulfate	380.133	$C_{19}H_{24}O_6S$
44.	10.602	Istamycin C	403.2724	$C_{18}H_{37}N_5O_5$
45.	11.251	Salmeterol	415.2746	$C_{25}H_{37}NO_4$
46.	11.5	13-Deoxycarminomycin	499.1877	$C_{26}H_{29}NO_9$
47.	11.59	Thermopsine	244.1573	$C_{15}H_{20}N_2O$
48.	12.449	Chalciporone	243.1622	$C_{16}H_{21}NO$
49.	14.01	N-Acetyl-Odemethylpuromycin	499.2243	$C_{23}H_{29}N_7O_6$
50.	14.202	Ganglioside GA2 (d18:1/20:0)	1120.7465	$C_{58}H_{108}N_2O_{18}$
51.	14.655	Anacyclin	271.1935	$C_{18}H_{25}NO$
52.	15.9	Lactosylceramide (d18:1/16:0)	861.6046	$C_{46}H_{87}NO_{13}$
53.	1.122	2,4-Dichlorotoluene	159.9841	$C_7H_6C_{12}$
54.	5.121	L-1,2,3,4-Tetrahydro-betacarboline-3-carboxyl	216.088	$C_{12}H_{12}N_2O_2$
55.	5.202	6''-Malonylastragalin	534.0957	$C_{24}H_{22}O_{14}$
56.	5.237	(-)-Epicatechin 3'-Oglucuronide	466.1087	$C_{21}H_{22}O_{12}$
57.	5.243	Isoferulic acid	194.0557	$C_{10}H_{10}O_4$
58.	5.747	3'-Methoxyfukiic acid	286.0669	$C_{12}H_{14}O_8$
59.	6.085	Caffeic acid 4-O-glucuronide	356.0718	$C_{15}H_{16}O_{10}$
60.	6.172	(±)-Glycerol 1,2-diacetate	176.0667	$C_7H_{12}O_5$
61.	6.227	Methacycline	442.1352	$C_{22}H_{22}N_2O_8$
62.	6.348	Flavine Mononucleotide (FMN)	456.1004	$C_{17}H_{21}N_4O_9P$
63.	6.386	Caffeic acid	180.0409	$C_9H_8O_4$
64.	6.439	Astragalin 7-rhamnoside	594.1553	$C_{27}H_{30}O_{15}$
65.	6.7	Kaempferol 3-rhamnoside 7- xyloside	564.146	$C_{26}H_{28}O_{14}$
66.	7.036	Phrymarolin I	488.13	$C_{24}H_{24}O_{11}$
67.	7.757	Phenylacetaldehyde	120.0568	C_8H_8O
68.	8.583	Flocoumafen	542.1665	$C_{33}H_{25}F_3O_4$
69.	8.649	Azelaic acid	188.1031	$C_9H_{16}O_4$
70.	9.283	methyl (+)-7-isojasmonate	224.14	$C_{13}H_{20}O_3$
71.	9.321	m-Hydroxybenzoic acid	138.0306	$C_7H_6O_3$
72.	11.128	5-Heptyltetrahydro-2-oxo-3- furancarboxylic acid	228.1348	$C_{12}H_{20}O_4$
73.	12.655	C.I. 14700	436.0419	$C_{18}H_{16}N_2O_7S_2$

Table 3: LC-MSMS analysis of the Ethanol extract of *Adhathoda vasica*.

Sl. No.	RT	Compound	Molecular weight	Molecular formula
1.	4.667	L-Tryptophan	204.0889	$C_{11} H_{12} N_2 O_2$
2.	4.814	Peganine	188.0947	$C_{11} H_{12} N_2 O$
3.	4.922	N-Acetyl-D-tryptophan	246.0991	$C_{13} H_{14} N_2 O_3$
4.	4.933	2-Amino-5-phenylpyridine	170.0837	$C_{11} H_{10} N_2$
5.	5.082	Meclizine	390.1826	$C_{25} H_{27} C_1 N_2$
6.	5.082	L-1,2,3,4-Tetrahydro-betacarboline-3-carboxylic acid	216.089	$C_{12} H_{12} N_2 O_2$
7.	5.099	(all-E)-Crocetin	328.1642	$C_{20} H_{24} O_4$
8.	5.487	Cyclo(L-Phe-L-Pro)	244.1202	$C_{14} H_{16} N_2 O_2$
9.	5.639	Maculosin	260.1154	$C_{14} H_{16} N_2 O_3$
10.	5.679	N-Acetylserotonin	218.1049	$C_{12} H_{14} N_2 O_2$
11.	6.084	Isopentenyl adenosine	335.1623	$C_{15} H_{21} N_5 O_4$
12.	6.307	Fluacrypyrim	426.1418	$C_{20} H_{21} F_3 N_2 O_5$
13.	6.515	Imazamethabenz	274.131	$C_{15} H_{18} N_2 O_3$
14.	6.574	2-Phenylethyl 3- methylbutanoate	206.1297	$C_{13} H_{18} O_2$
15.	6.841	Kaempferol 3-rhamnoside 7- xyloside	564.1457	$C_{26} H_{28} O_{14}$
16.	7.095	Gibberellin A105	330.147	$C_{19} H_{22} O_5$
17.	7.099	N6-cis-p-Coumaroylserotonin	322.131	$C_{19} H_{18} N_2 O_3$
18.	7.826	Tropisetron	284.1533	$C_{17} H_{20} N_2 O_2$
19.	7.939	WIN VI	314.1627	$C_{18} H_{22} N_2 O_3$
20.	7.98	Tryptophyl-Alanine	275.1287	$C_{14} H_{17} N_3 O_3$
21.	8.073	Hematoporphyrin	598.2886	$C_{34} H_{38} N_4 O_6$
22.	8.148	Famciclovir	321.147	$C_{14} H_{19} N_5 O_4$
23.	8.287	Arginyl-Methionine	305.1518	$C_{11} H_{23} N_5 O_3 S$
24.	8.37	Caribenolide I	624.3617	$C_{33} H_{52} O_{11}$
25.	8.427	Aurasperone E	588.1726	$C_{32} H_{28} O_{11}$
26.	8.773	1-Octen-3-yl primeveroside	422.2139	$C_{19} H_{34} O_{10}$
27.	8.807	Etoposide	588.173	$C_{29} H_{32} O_{13}$
28.	8.81	Istamycin A1	417.2588	$C_{18} H_{35} N_5 O_6$
29.	9.156	Ethylketocyclazocine	299.1833	$C_{19} H_{25} N O_2$
30.	9.24	Isopentenyl adenosine	335.1628	$C_{15} H_{21} N_5 O_4$
31.	9.432	Goyaglycoside c	662.4323	$C_{38} H_{62} O_9$
32.	9.544	Brevianamide B	365.1742	$C_{21} H_{23} N_3 O_3$
33.	10.877	Methionyl-Tryptophan	335.1274	$C_{16} H_{21} N_3 O_3 S$
34.	10.913	Oleandolide	386.235	$C_{20} H_{34} O_7$
35.	11.132	Dihydrodeoxystreptomycin	567.2888	$C_{21} H_{41} N_7 O_{11}$
36.	11.168	C16 Sphinganine	273.2671	$C_{16} H_{35} N O_2$
37.	13.096	N1-(2-Methoxy-4- methylbenzyl)-n2-(2-(5-methylpyridin-2- yl)ethyl)oxalamide	341.1755	$C_{19} H_{23} N_3 O_3$
38.	13.155	Campesteryl ferulate	576.419	$C_{38} H_{56} O_4$
39.	13.314	Methyl 2-furoate	126.0339	$C_6 H_6 O_3$
40.	13.501	Gingerglycolipid A	676.3658	$C_{33} H_{56} O_{14}$
41.	13.658	Sterebin A	310.2141	$C_{18} H_{30} O_4$

Sl. No.	RT	Compound	Molecular weight	Molecular formula
42.	14.047	Armillarin	414.2048	$C_{24} H_{30} O_6$
43.	14.235	Isomytiloxanthin	598.4024	$C_{40} H_{54} O_4$
44.	14.957	all-trans-heptaprenyl diphosphate	654.3818	$C_{35} H_{60} O_7 P_2$
45.	15.012	6-pentadecyl Salicylic Acid	348.2663	$C_{22} H_{36} O_3$
46.	15.315	19-Noretiocholanolone	276.2087	$C_{18} H_{28} O_2$
47.	15.862	Lactosylceramide (d18:1/16:0)	861.605	$C_{46} H_{87} N O_{13}$
48.	16.012	Majoroside F3	800.4889	$C_{42} H_{72} O_{14}$
49.	16.802	Misoprostol	382.2718	$C_{22} H_{38} O_5$
50.	17.458	Methyl 2-furoate	126.0337	$C_6 H_6 O_3$
51.	17.504	Phthalic acid Mono-2- ethylhexyl Ester	278.1513	$C_{16} H_{22} O_4$
52.	20.085	Harderoporphyrin	608.2628	$C_{35} H_{36} N_4 O_6$
53.	1.353	pHydroxybenzyldesulphoglucosi	345.0891	$C_{14} H_{19} N O_7 S$
54.	5.124	L-1,2,3,4-Tetrahydro-betacarboline-3-carboxylic	216.088	$C_{12} H_{12} N_2 O_2$
55.	6.529	beta-D-Glucopyranosyl-11- hydroxyjasmonic acid	388.1718	$C_{18} H_{28} O_9$
56.	6.563	Diacetylfusarochromanone	376.1613	$C_{19} H_{24} N_2 O_6$
57.	6.572	S-Furanopetasitin	432.1985	$C_{24} H_{32} O_5 S$
58.	6.792	Kaempferol 3-rhamnoside 7- xyloside	564.1439	$C_{26} H_{28} O_{14}$
59.	7.194	Phrymarolin I	488.13	$C_{24} H_{24} O_{11}$
60.	7.906	Licocoumarin A	406.1711	$C_{25} H_{26} O_5$
61.	7.914	Ethyl 7-epi-12- hydroxyjasmonate glucoside	416.2026	$C_{20} H_{32} O_9$
62.	7.983	Austalide C	574.234	$C_{30} H_{38} O_{11}$
63.	8.019	Brassica napus nonfluorescent chlorophyll catabolite 3	630.2705	$C_{34} H_{38} N_4 O_8$
64.	8.027	4Z,15E-Bilirubin IXa	584.2653	$C_{33} H_{36} N_4 O_6$
65.	8.149	Funebrine	348.1659	$C_{18} H_{24} N_2 O_5$
66.	8.158	17beta-Hydroxy-4- mercaptoandrost-4-en-3-one 4-acetate 17-propionate	418.2184	$C_{24} H_{34} O_4 S$
67.	8.812	Cinalukast	412.1835	$C_{23} H_{28} N_2 O_3 S$
68.	10.286	Corchorifatty acid F	328.2227	$C_{18} H_{32} O_5$
69.	10.807	9,10-Dihydroxy-12,13- epoxyoctadecanoate	330.239	$C_{18} H_{34} O_5$
70.	12.648	C.I. 14700	436.0416	$C_{18} H_{16} N_2 O_7 S_2$
71.	15.912	Vinaginsenoside R1	842.5082	$C_{44} H_{74} O_{15}$
72.	15.966	Mabioside D	798.4827	$C_{42} H_{70} O_{14}$
73.	16.037	PPA(16:0/18:1(9Z))	754.4565	$C_{37} H_{72} O_{11} P_2$
74.	16.124	Torvoside E	770.4513	$C_{40} H_{66} O_{14}$
75.	16.212	Torvoside D	726.4261	$C_{38} H_{62} O_{13}$
76.	16.299	Madlongiside C	636.3946	$C_{35} H_{56} O_{10}$
77.	16.471	26-Glucosyl-1,3,11,22-tetrahydroxyergosta-5,24-dien26-oate	638.3739	$C_{34} H_{54} O_{11}$
78.	18.615	Integerressine	554.2896	$C_{33} H_{38} N_4 O_4$
79.	18.719	5β-Cyprinolsulfate	532.3082	$C_{27} H_{48} O_8 S$
80.	19.175	α-Linolenic Acid	278.2229	$C_{18} H_{30} O_2$

Because the peaks were prominent, the predominant compounds were present in the ethanol extract. antioxidant activity was measured in the ethanolic extract. Antioxidant activity results showed that the *Adhathoda vasica* ethanolic extracts exhibited a DPPH scavenging effect in a concentration-dependent manner. Similarly, the ABTS assay showed that the ethanol extract also exhibited antioxidant activity, with higher concentrations showing increased percentage inhibition compared to lower concentrations. At certain concentrations (25 µg and 75 µg), the antioxidant activity of the ethanol extract was similar to or exceeded the antioxidant activity of naringenin. The results of the anti-oxidant assays are in concordance with the study done by Mamta P and Sujata[26] who stated that the ethanolic extract of *Adhatoda vasica* showed high antioxidant activity with cytoprotective potential in cell culture. The presence of phenolic compounds could be a possible reason for this antioxidant activity.[27] These Phenolic compounds are a class of antioxidant agents that act as free radical terminators.[28] Hence, we believe that the potential radical scavenging and antioxidant properties of *Adhatoda vasica* are due to the presence of total phenols and tannins.

CONCLUSION

Adhatoda vasica has been used in traditional medicine for centuries, owing to its diverse therapeutic benefits. This study aimed to identify the bioactive compounds present in both polar and non-polar leaf extracts of *Adhatoda vasica*, which are thought to contribute to its medicinal properties. The analysis revealed the presence of several bioactive compounds including saponins, phenolic flavonoids, tannins, flavonoids, phytosterols, terpenoids, phenols, and steroids. These compounds are believed to be responsible for the therapeutic activities of plants. These findings suggest that these bioactive compounds may be valuable in the development of natural-based drug formulations. The antioxidant activity of the extracts, attributed to compounds such as flavonoids, terpenoids, and phenols, was particularly notable. The high antioxidant potential of the ethanolic extract is significant because it can inhibit or slow the progression of oxidative stress-related illnesses. Moreover, the results provide a foundation for further research on *Adhatoda vasica*. Additional *in vitro* studies are necessary to validate the pharmacological activities of these bioactive compounds and explore their potential in treating various health conditions, including cancer.

ACKNOWLEDGEMENT

None.

ABBREVIATIONS

ABTS: 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **LC-MS-MS:** Liquid Chromatography with tandem mass spectrometry; ROS: Reactive Oxygen Species; **GAE:** Gallic Acid Equivalent; **DW:** Dry Weight; **CE:** Catechin Equivalent; **PBS:** Phosphate-Buffered Saline; **RT:** Retention Time.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

H.F.K-Conceptualization, Methodology, Formal analysis. Investigation, Writing - Original Draft J.M.-Conceptualization, Methodology, Formal analysis, Supervision,Writing - Review & Editing A.K.P.- Formal analysis, Supervision,Writing - Review & Editing.

REFERENCES

1. Samy RP, Thwin MM, Gopalakrishnakone P, Ignacimuthu S. Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamilnadu, India. J Ethnopharmacol. 2008;115(2):302-12.
2. Pham-Huy LA, H He and Pham-Huy C. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci. 2008;4(2):89-96.
3. Amin AH, Mehta BR. Bronchodialator alkaloid (vasicinone) from *Adhatoda vasica*. Nature. 1959;184:1317.
4. Mark E. Sorrells Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. Food Chemistry. 2010;119(1):249-57.
5. Tomohiro Sawa. Mayumi Nakao. Takaaki Akaike. Kanji Ono. Hiroshi Maeda. Alkylperoxyl Radical-Scavenging Activity of Various Flavonoids and Other Phenolic Compounds: Implications for the Anti-Tumor-Promoter Effect of Vegetables. J. Agric. Food Chem. 1999;47(2):397-402.
6. Srivastava N, Srivastava A, Banerjee A, Nivasarkar M. Antiulcer activity of *Adhatoda vasica* Nees. J Herb Pharmacother. 2006;6(2):43-49.
7. Duraipandiyan. V *et al.* Antimicrobial, Antioxidant, and CytotoxicProperties of Vasicine Acetate Synthesized from Vasicine Isolated from *Adhatoda vasica* L. BioMed Res. Int. 2015; 7 doi: 10.1155/2015/727304.
8. Vinothapooshan G. and Sundar K. Hepatoprotective activity of *Adhatoda vasica* leaves against carbontetrachloride induced toxicity. Pharmacologyonline. 2010;2:551-8.
9. Duh PD. Antioxidant activity of burdock (*Arctium lappa* Linné): Its scavenging effect on free-radical and active oxygen. J Amer Oil Chem Soc. 1998;75:455-61.
10. Candan F *et al.* Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). J Ethnopharmacol. 2003;87(2-3):215-20
11. ***11.*** Sadasivam, S. Biochemical methods; New Age International: New Delhi, India. 1996; 978-81.
12. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965;16:144-58.
13. Fadda, A.; Mulas, M. Chemical changes during myrtle (*Myrtus communis* L.) fruit development and ripening. Sci. Hort. 2010;125:477-85.
14. Katalinic, V *et al.* Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry. 2006;94(4):550-57.
15. Bajpai, V. K., Agrawal, P., Bang, B. H., and Park, Y. H. Phytochemical analysis, antioxidant and antilipid peroxidation effects of a medicinal plant,*Adhatoda vasica*.*Frontiers in Life Science*, 2015;*8*(3):305-12.
16. Vankata Bhaskara RK, Minki M, Amie P, Loganathan K, Gaurav K. Phytochemical Composition, Antioxidant, Antimicrobial and Cytotoxic Potential of Methanolic Extracts of *Adhatoda vasica* (Acanthaceae). Research Journal of Pharmacy and Technology, 2013;6(9):1004-9.
17. Mohammad KA, Seema B, Rajesh Y. Phytochemical Screening and Antioxidant activity of extract of different parts of *Adhatoda vasica*. Research Journal of Pharmacy and Technology, 2019;12(2):5699-705.
18. Nandhini S, Ilango K. Comparative Study on Pharmacognostical, Phytochemical Investigations and Quantification of Vasicine Content in the Extracts of *Adhatoda vasica* Nees and *Adhatoda beddomei* CB Clarke. Pharmacogn J. 2020;12(4):884-96

19. Claeson U P *et al. Adhatoda vasica*: a critical review of ethnopharmacological and toxicological data. J Ethnopharmacol. 2000;72(1-2):1-20.
20. Okarter, N. and Liu, R.H. Health Benefits of Whole Grain Phytochemicals. Crit Rev Food Sci Nutr, 2010; 50 (3): 193-208.
21. Kripasana K. and Xavier J. Phytochemical analysis and antioxidant activity of leaf extracts of some selected plants of the family Acanthaceae. Plant Science Today.2020;7(2):264-74.
22. Amarowicz R., Pegg R., Rahimi-Moghaddam P., Barl B., Weil J. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 2004;84:551-562. doi: 10.1016/S0308-8146(03)00278-4
23. Soobrattee M.A., Neergheen V.S., Luximon-Ramma A., Aruoma O.I., Bahorun T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat. Res.-Fund. Mol. Mutagen. 2005;579:200-13.
24. Molole, G.J., Gure, A. and Abdissa, N. Determination of total phenolic content and antioxidant activity of *Commiphora mollis* (Oliv.) Engl. resin. BMC Chemistry, 2022;16:48. https://doi.org/10.1186/s13065-022-00841-x
25. Ayele, D.T., Akele, M.L. and Melese, A.T. Analysis of total phenolic contents, flavonoids, antioxidant and antibacterial activities of *Croton macrostachyus* root extracts. BMC Chemistry, 2022;16:30. https://doi.org/10.1186/s13065-022-00822-0.
26. Mamta P *et al.* Antioxidant and free radical scavenging potential of ethanolic fraction of *Adhatoda vasica* in A549 cell line. Asian J Pharm Clin Res.2015;8(6):244-9.
27. Kokati Vankata Bhaskara Rao *et al.* Phytochemical Composition, Antioxidant, Antimicrobial and Cytotoxic Potential of Methanolic Extracts of *Adhatoda vasica* (Acanthaceae). Research Journal of Pharmacy and Technology. 2013;6(9): 997-1002.
28. Shahidi F *et al.* Phenolic antioxidants. Crit Rev Food Sci Nutr. 1992;32(1):67-103.

Cite this article: Kamal HF, Mahendra J, Pandurangan AK. Extraction, Phytochemical Analysis, and Antioxidant Activity of *Adhatoda vasica* Leaves. Indian J of Pharmaceutical Education and Research. 2026;60(3s):s1203-s1214.